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EMBRYONIC DIAPAUSE IN *TIPULA SIMPLEX* AND THE ACTION OF PHOTOPERIOD IN ITS TERMINATION (DIPTERA: TIPULIDAE)

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Most crane fly larvae are stream dwelling or live in areas where there is a constant supply of moisture (Alexander, 1967), but there are reports of species thriving in fairly dry soil on almost all continents (Alexander, 1920; 1931). A few of these species, including *Tipula simplex* of the Central Valley of California, live through cyclic droughts (Hartman and Hynes, 1977).

Most crane fly eggs have a development time of one to three weeks, depending on ambient temperature, and require constant moisture (Hemmingsen, 1956; Byers, 1961; Hynes, 1963; Hartman, 1966). The eggs of *Tipula simplex* have a stadium of 8 months and require six to seven months of drought, two periods of moisture, separated by a second drying period, under a long scotophase before they will hatch (Hartman and Hynes, 1977; Hartman and Hynes, in press).

Based on the difference in environmental conditions in which they live, egg stadium length, and moisture and photoperoid regimen necessary to induce hatching between *Tipula simplex* and other tipulids, we have postulated a summer dormancy for *T. simplex*. Mansingh (1971) classifies dormancies into quiescence, oligopause or diapause based on physiological characteristics of the dormant stage and relates the type to ecological characteristics of the environment.

We report here on tests that we have run to determine whether the dormancy in *Tipula simplex* eggs is quiescence, oligopause, or diapause. Determinations were: time of the onset of dormancy; amount of embryonic development occurring during dormancy; time of refractory and activation phases; oxygen consumption rates; effect of photoperiod on dormancy termination.

Table 1. Percent hatch of eggs under photoperiods of varying lengths.

N of eggs	Month of moistening	Photoperiod	T	% Hatch
400	December	6L:6D	12	29 a
400	December	18L:6D	24	5 b
400	December	30L:6D	36	22 a
400	December	42L:6D	48	9 b

Numbers followed by the same letter are not significantly different ($P < .05$) according to Duncan's multiple range test.

Materials and Methods

Collection of eggs.—The *Tipula simplex* eggs were collected in the field in Tulare County, California, using previously described techniques (Hartman and Hynes, in press).

Sectioning and staining.—One hundred eggs were fixed monthly from Apr. to Sept. in Carnoy and Lebrun's fluid (Galigher and Kozloff, 1964), imbedded according to the n-butyl methacrylate method of Woodring and Cook (1962) as modified by Smith and Hynes (1966), cut at 10 μ on an AO rotary microtome, stained with acid fuchsin and Mallory's triple, and destained with 2% phosphomolybdic acid (Galigher and Kozloff, 1964).

Induction of hatching.—To induce hatching, eggs were transferred onto filter paper in a water-tight container 9 cm in diameter. The filter paper was moistened with 1.6 ml of distilled water and placed at 15° at a 10L:14D photoperiod (light phase = 0.76 lux). After two weeks, the lids were removed for one day, allowing the paper to dry. After an additional week, the paper was remoistened with 1.6 ml of distilled water and the lid was replaced. Hatches were counted twice weekly. Four replicates were used for each test.

To study the timing of the onset of dormancy, the refractory, and the activation phases, we attempted to induce hatching in 400 eggs every month for a year, using the methods described above.

To study the effect of photoperiod, eggs were treated as above except that they were dried until October, and when moistened, were placed at 10L:14D; 12L:12D; 14L:10D; or 16L:8D for the duration of the test.

To determine the mechanism of photoperiod interpretation, embryos were treated to induce hatching in December, but were placed in a photoperiod of 6L:6D, 18L:6D, 30L:6D, or 42L:6D.

Oxygen consumption.—500 eggs were placed on filter paper in each of ten 15 ml Gilson flasks. 10% KOH was added to the side arm of each flask to absorb CO_2. The eggs were dried and moistened in the proper regimen to induce hatching. Oxygen consumption was measured with a Gilson Respirometer (loaned by the J. G. Boswell Company).

Diapause termination.—The eggs of *Tipula simplex* have a sclerotized chorion, which makes it impossible to view embryonic development *in vivo*. The oxygen consumption rate of each egg is too low to use it as a quantitative measure of the percent of eggs which break diapause. We were forced to use percent of eggs hatching as a measure of diapause termination, which, due to embryonic mortality, will underestimate consistently the number of eggs which terminate diapause.

Results

Time of the onset of dormancy.—Eggs enter dormancy within one month of oviposition, even though photoperiod is adjusted to a 10L:14D condition. The refractory period extends from the time of ovipoisiton (March) until August. From August through January they are in the activation phase, then in February, they return to the refractory phase.

Embryonic development during dormancy.—Embryos which had been collected in the field and stored without moisture remained in the precleavage, or what Johanssen and Butt (1941) call the fusion nucleus stage. Development does not resume until the eggs are moistened in the fall. This is the earliest recorded dormancy condition in any insect. Lees (1955) states that "no species is known in which diapause supervenes before the formation of the blastoderm." A survey of the literature since 1955 did not reveal any other cases of diapause at this early stage of development. Although Iba and Inoue (1972) indicate that the egg of the rhombic-marked leaf hopper, *Hishimonus stellatus,* entered diapause immediately after oviposition, they do not indicate the stage of embryonic development at which diapause occurs.

Oxygen consumption rates.—Oxygen consumption varied tremendously with variations in the environmental conditions for hatching. During the summer drought, O_2 consumption was measured at 1.2 μl/100 eggs/hr (S.E. = 0.4). Twenty-four hours after moistening the oxygen consumption rate had risen to 4.0 μl/100 eggs/hr (S.E. = 0.2).

Interpretation of day length/night length.—Previous results indicate that a scotophase of 12–14 hours is most effective in inducing diapause termination. Two major models have been proposed to explain how living things time the scotophase. In the hourglass model, the length of night is measured by accumulation of some unknown metabolite, which is destroyed during the day. In the oscillator model, circadian clocks are involved (Pittendrigh, 1972). If circadian oscillations are involved, then the eggs should break dormancy and hatch whenever darkness occurs in the first and third quarters of a 24 hour day, whether the darkness is in both quarters in the same 24 hour period or not. If the hourglass system is working, then eggs should break dormancy and hatch when the minimum scotophase is coupled with a light requirement of any length above minimum. That is, if an oscillator system is involved, then the eggs should break dormancy and hatch under a light regimen of 12L:12D, 6L:6D, or 30L:6D. If the hourglass system is involved, then 12L:12D is the only light regimen which will terminate diapause.

The results, shown in Table 1, indicate that *Tipula simplex* embryos break dormancy whenever the scotophase occurs during the first and/or third quarter of a 24 hour day, thus indicating that the biological clock in *Tipula simplex* eggs is an oscillator system.

Discussion

Mansingh (1971) characterizes dormancy on the basis of both physiological and ecological characteristics. The physiological characteristics of diapause described by Mansingh (1971) include: entrance into dormancy well before the appearance of the adversity; growth arrest; complex biochemical changes; and a refractory period at least half the length of the normal dormancy period. *Tipula simplex* eggs enter dormancy in the fusion nucleus stage, which occurs soon after fertilization. This stage is reached three months before the onset of the adversity (June in Tulare County). The growth arrest observed is characteristic of diapause but may also be characteristic of oligopause (Mansingh, 1971). Complex biochemical changes associated with the termination of diapause have been characterized for *T. simplex* eggs only by changes in oxygen consumption rates so far. *Tipula simplex* eggs have the refractory period extended from March to August, which is more than half of the normal dormancy period (March to October). Therefore on the basis of the physiological data, *Tipula simplex* dormancy may be classified as true diapause.

Mansingh's work (1971) has been criticized by Thiele (1973), for failure to separate diapause into parapause (genetically fixed stage of development with no clear phase of induction) and eudiapause (facultative diapause with a clear induction phase), and for his attempts to correlate dormancy with distribution.

Mansingh (1971) states that an insect with a diapause stage has its range confined to an area of extreme adversity, and this adversity is long term and cyclic. Insects with an oligopause stage are more wide ranging and the adversity is not as extreme, but is cyclic. In quiescent stages the adversity is unanticipated. *Tipula simplex* has a geographical distribution from Contra Costa County north to Sacramento County, and a separate population in Tulare County, all in California (Alexander, 1967). Within this geographical range, it is confined to those unirrigated lands which undergo seasonal drought from May to Oct. At these times soil moisture is as low as 0.2% and soil temperature as high as 50°C (Hartman and Hynes, 1977). The habitat of *Tipula simplex* diapause neatly fits into the extreme cyclic environment predicted by Mansingh for diapause conditions.

The oscillator system of biological clock is much more common than the hourglass system (Beck, 1968). Pittendrigh (1971) indicates that there are two possible oscillator systems, an external coincidence and an internal coincidence system. Whether the system in *T. simplex* is an external or an internal coincidence remains to be determined.

Conclusions

On the basis of ecological and physiological characteristics of dormancy in *Tipula simplex* eggs, we postulate that summer dormancy is a true diapause condition. Work is continuing on the characterization of the condition existing during the obligatory drying period between the first and second fall moistenings. A long scotophase is essential for termination of diapause. The biological clock involved is an oscillator type.

Literature Cited

Alexander, C. P. 1967. The crane flies of California. Bull. Calif. Insect Surv. Vol. 8. Univ. Calif. Press, Berkeley and Los Angeles, 269 p.

Beck, S. D. 1968. Insect Photoperiodism. Academic Press, New York, 288 p.

Byers, G. W. 1961. The crane fly genus *Dolichopeza* in North America. Univ. Kans. Sci. Bull., 42(6):665–924.

Galigher, A. E., and E. N. Kozloff. 1964. Essentials of practical microtechniques. Lea & Febiger, Philadelphia, 484 p.

Hartman, M. J. 1966. A study of organogenesis in the egg of *Erioptera* (*Trimicra*) *pilipes* (Tipulidae: Diptera). Unpublished thesis, Cal. Poly., San Luis Obispo.

Hartman, M. J., and C. D. Hynes. 1977. The biology of the range crane fly, *Tipula simplex* (Diptera: Tipulidae). Pan-Pacific Entomol., 53:118–123.

Hartman, M. J., and C. D. Hynes. Environmental factors affecting the hatching success of *Tipula simplex* eggs (Diptera: Tipulidae). Submitted to Pan-Pacific Entomol.

Hemmingsen, A. M. 1965. The lotic crane fly *Tipula saginata* Bergroth and the adaptive radiation of the Tipulinae, with a test of Dyar's Law. Vidensk Medd. Dan Naturhist. Foren., 128:93–150.

Hynes, C. D. 1963. Description of the immature stages of *Cryptolabis magnistyla* Alexander. Pan-Pacific Entomol., 39:255–260.

Iba, M., and S. Inoue. 1972. On the diapause in hibernating eggs of the rhombic-marked leafroller, *Hishimonus sellatus* Uhler. J. Seric. Sci. Jpn., 41(5):365–370.

Johannsen, O. A., and F. H. Butt. 1941. Embryology of insects and myriapods. McGraw-Hill, New York, 462 p.

Lees, A. D. 1955. The physiology of diapause in arthropods. Cambridge Univ. Press, 150 p.

Mansingh, A. 1971. Physiological classification of dormancies in insects. Canadian Entomol., 103:983–1009.

Pittendrigh, C. S. 1972. Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. Proc. Nat. Acad. Sci. USA, 69:2734–2737.
Smith, H. G., and C. D. Hynes. 1966. Modifications of the n-butyl methacrylate imbedding method as applied to insect larval head capsules. Ann. Entomol. Soc. Amer., 59:230.
Thiele, H. U. 1973. Remarks about Mansingh's and Müller's classifications of dormancies in insects. Canad. Entomol., 105:925–928.
Woodring, J. P., and E. F. Cook. 1962. The internal anatomy, reproductive physiology and molting process of *Ceratozetes cisalpinus* (Acarina: Orobatei). Ann. Entomol. Soc. Amer., 55:164–181.

Footnote

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BOOK REVIEW

Ronald A. Russo. 1979. *Plant Galls of the California Region.* The Boxwood Press, Pacific Grove, California. xi + 203 pages, 190 text figures, bibliography, taxonomic and subject indexes. $8.95, paper.

There is at long last a book to aid in identifying plant galls induced by various biological agents in California. It is simple enough for the beginner and yet complete enough to be of value to serious students of the various groups of gall inducers. It reflects 10 years of the author's fascination with the intricate biochemical relationships between the host plant and the gall inducers and the great beauty of the galls they cause. The only other comparable prior works were either too broad in geographic range or too specialized. The paperback book is small enough and the simple line drawings are in most cases adequate to identify the galls in the field.

Except for an editorial prerequisite to use the terms "larvas" and "pupas" which is frustrating to the author and some readers, the natural history discussions are enjoyably informative. I had to take turns getting to read my own copy. Russo discusses most of the conspicuous galls and some of the obscure forms. Information on additional species is being compiled for expanded treatment of at least the cynipid gall wasps, which will make the second edition even more valuable.

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